

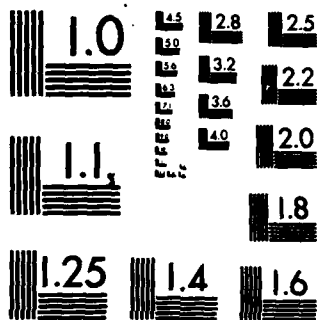
ACUTE SYSTEMIC CHANGES IN BLOOD CELLS PROTEINS  
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ENVIRONMENTAL MEDICINE NATICK MA J J GADAROWSKI ET AL.  
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the plasma coagulation tests were a prolonged partial thromboplastic time, an accelerated prothrombin time and increased activities of Factors VII, IX, X, and XI. In mild frostbitten rabbits there were a prolonged partial thromboplastin time and an increased activity of Factor VII. No changes in fibrinolysis were seen in either group of rabbits. Platelet aggregation, studied only in the severely frostbitten rabbits, showed a change only by an increase in the slope of the collagen induced platelet aggregation. The blood changes observed in the rabbit model are different than those reported in human frostbite cases. No disseminated intravascular coagulation was apparent in the rabbit model after frostbite injury.

Acute Systemic Changes in Blood Cells,  
Proteins, Coagulation, Fibrinolysis, and Platelet Aggregation  
After Frostbite Injury in the Rabbit<sup>\*,\*\*</sup>

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## ANIMAL RESEARCH

\*In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

\*\*The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

## INTRODUCTION

Damage to the endothelium of the blood vessels and thrombosis have been observed both in human (10) and in experimental cold injury (6,27). Evidence suggests that injury to the microvascular system plays a decisive role in the development of tissue destruction in frostbite (24,34). Weatherley-White et al. (40) showed this fact when they were able to have frostbitten skin survive when it was transplanted to a normal tissue bed, whereas normal skin necrosed when it was transplanted to a frostbitten tissue bed.

Although neural and endothelial cells are probably the most sensitive to cold injury (1), very few studies have dealt with changes in blood coagulation, fibrinolysis, or platelet aggregation in frostbite injury. Leep et al. (25) measured a prolonged prothrombin time in low temperature exposed rats. Barkagan and Plotnikov (2) stated that in 260 patients with acute frostbite injury, they found an increase in platelet aggregation and an activation of the intrinsic and extrinsic blood coagulation pathways. In 15 human high altitude frostbite cases Chohan et al. (8) concluded that disseminated intravascular coagulation occurred because they found increased fibrin degradation products; decreased fibrinogen concentration, platelet count, and antithrombin III activity; and a prolonged euglobulin lysis time. Murazyan et al. (28) stated that hypercoagulation existed in frostbite injury, but the fibrinogen concentration increased rather than decreased after injury.

The purpose of this study was to measure acute systemic changes in blood cell counts, certain plasma proteins, plasma coagulation times, plasma clotting factors, fibrinolysis, and platelet aggregation in rabbits with different degrees of frostbite injury. The rabbit was chosen, since it has been used extensively by others in studying the nature and treatment of frostbite injury (1,3,12,15).

## MATERIALS AND METHODS

Animals. Thirty-seven New Zealand white female rabbits weighing 8-12 lbs. were used. They were divided into 3 groups: one group for plasma protein changes, blood clotting factor assays, and clotting assays; a second group for fibrinolysis; and a third group for platelet aggregation. The first and second groups were frozen either to  $-20^{\circ}\text{C}$  or to  $+5^{\circ}\text{C}$ ; the third group was frozen only to  $-20^{\circ}\text{C}$ .

Injury Production. The left hind foot was shaved before freezing. A sterilized 23 gauge needle microprobe, type MT-23/5 (Bailey Instruments, Inc; Saddle Brook, NJ) was inserted aseptically into the middle of the ventral musculature of the metatarsal region of the foot at a depth equidistant between the dorsal and ventral surfaces of the foot. The tip of the microprobe was positioned 5 millimeters from the distal ends of the second and third metatarsal bones. The intramuscular length of the microprobe was about 1 centimeter. The foot was covered with a sterilized surgical glove to prevent fluid contact during freezing and rewarming. The foot was immersed up to the prominent tuberosity of the 5th metatarsal bone in a cooling bath (FTS Systems, Inc; Stone Ridge, NY) containing 95% methanol maintained at a temperature of  $-40^{\circ}\text{C}$ . It was rewarmed in a similar bath containing water at  $+37^{\circ}\text{C}$ . Temperature was monitored simultaneously from the skin, rectum, room, and cooling and rewarming baths by using at each site a general purpose/rectal thermometer probe, type RET-1 (Bailey Instruments, Inc.). All temperatures were monitored on a Numatron<sup>R</sup> (Leeds and Northrup; North Wales, PA) and recorded on a PDP 11/40 computer.

Severe frostbite injury was produced by cooling the foot until the microprobe recorded an internal temperature of  $-20^{\circ}\text{C}$ , whereas mild frostbite injury was produced by cooling the foot to an internal temperature of  $+5^{\circ}\text{C}$ .



When the desired temperature was reached, the foot was immediately rewarmed to  $+35^{\circ}\text{C}$ , and the microprobe was removed.

Blood Collection. Prior to each blood collection each rabbit was given 5 mg of acepromazine maleate (Fort Dodge Laboratories, Inc.; Fort Dodge, IA) intramuscularly for sedation and 25 mg of Nembutal<sup>R</sup> (Abbott Laboratories; North Chicago, IL) intravenously for anesthetization. On the day of freezing it was necessary to use 100 to 125 mg of Nembutal<sup>R</sup> to ensure adequate anesthetization. 4.5 ml of blood was obtained from the central ear artery using a Venoject<sup>R</sup> vacuum blood collection tube (Kimble-Terumo, Inc.; Elkton, MD) containing 0.5 ml of 3.8% sodium citrate. The blood was immediately removed from the collection tube and transferred into a 12 x 75 mm polypropylene test tube. Serum for the fibrin degradation product assay was obtained by collecting 2 ml of blood into a vacuum tube containing soybean trypsin inhibitor and bovine thrombin (Wellcome Diagnostic; Research Triangle Park, NC). Platelet poor plasma was obtained by centrifuging the blood at  $4^{\circ}\text{C}$  at 3200 G for 10 minutes; and platelet rich plasma, at room temperature at 126 G for 5 minutes. Half ml aliquots of the platelet poor plasma and serum were frozen in liquid nitrogen and were stored at  $-40^{\circ}\text{C}$ .

Blood Sampling Schedule. For the studies on plasma protein, blood cells, clotting tests, clotting factor assays, and fibrinolysis, control blood samples were taken for three days during the first week. In the second week the microprobe was inserted into the foot kept at room temperature. After 45 minutes the microprobe was removed, and blood samples were taken 1 hour, 24 hours, 48 hours, and 72 hours after removal. In the third week control blood samples from the same rabbit again were taken for three days. In the fourth week the rabbit foot was frozen, and blood samples were taken at the same time intervals as for the second week. Each rabbit served as its own control.

For the platelet aggregation studies control blood samples were taken three times in the first week. In the second week the foot was frozen, and blood samples were obtained 1 hour, 24 hours, 48 hours, and 72 hours after rewarming.

Assays. Hematocrit and erythrocyte, leukocyte, and platelet counting followed routine procedures. The platelet count was done on a hemocytometer using a phase contrast microscope, whereas the erythrocyte and leukocyte counts were done on a Coulter<sup>R</sup> counter, model ZBI (Coulter Electronics; Hialeah, FL).

Plasma hemoglobin was assayed according to Harboe (19). Plasma albumin concentration was determine with the albumin kit, number 630, from Sigma Chemical Co. (St. Louis, MO). Rabbit albumin (Sigma) was used as the standard. Total serum globulin concentration was assayed by the globulin kit, number 560, from Sigma.

Factor XII, XI, X, IX, VIII, and VII deficient plasmas, 0.025M calcium chloride, automated simplastin reagent, platelet factor 3 reagent plus activator, and the procedures for the prothrombin time, activated partial thromboplastin time, and the clotting factor assays were obtained from General Diagnostics (Morris Plains, NJ). Factor II and VII deficient plasma, Russell's viper venom in cephalin solution, and the procedure for the Factor II assay were obtained from Sigma. Factor I was assayed according to Sirridge (36).

Antithrombin III was assayed according to Bick et al. (4) except bovine fibrinogen, type 1, from Sigma was used, and the plasma was defibrinated with reptilase<sup>R</sup> -R (Abbott Laboratories; North Chicago, IL) according to the manufacturer's instructions.

Fibrin/fibrinogen degradation products were determined by the paracoagulation method using the Dade<sup>R</sup> Data-Fi<sup>R</sup> protamine sulfate kit (American Scientific Products; McGaw Park, IL), by the staphylococcal clumping method using the fibrin/fibrinogen degradation product kit, number 850, from

Sigma and by the tanned red cell hemagglutination-inhibition method using the Wellcome FDP Kit (Wellcome Diagnostics).

Due to the high concentration of inhibitors against fibrinolysis in rabbit plasma (22), a euglobin solution was used in the plasminogen assay. This was prepared according to Iatridis and Ferguson (23), except the buffer used to redissolve the euglobulins was 0.1 M tris (hydroxymethyl) aminomethane (TRIS) at pH 7.5, and the euglobulins were precipitated at pH 6.0 (21). To 0.05 ml of the euglobulin solution was added 0.05 ml of TRIS buffer. Then 0.1 ml of urokinase (Calbiochem-Behring Corp.; San Diego, CA) at 500 Plough units/ml dissolved in distilled water was added. The solution incubated immediately at 37°C for 5 minutes. Fibrinogen uncontaminated with plasminogen was used in the assay. This was prepared according to Deutsch and Mertz (13), except in the purification procedure fibrinogen type 1 was used in place of plasma, and the buffer was 0.1 M TRIS. The remaining part of the plasminogen assay followed the procedure of Uete et al. (39). A standard curve was prepared in which various volumes of tyrosine at 1.2 mg/ml dissolved in 0.1 N hydrochloric acid was added to 0.1 M TRIS buffer to equal a final volume of 0.2 ml. To this was added 2.0 ml of 0.1 M TRIS buffer and 1.0 ml of 15% trichloroacetic acid. Results of the plasminogen assay are expressed as nmoles of tyrosine liberated  $\text{min}^{-1} \text{ml}^{-1}$  of plasma sample.

The euglobulin lysis time was done according to Iatridis and Ferguson (23) except defibrinated plasma, as prepared above, was used and the euglobulins were precipitated at pH 6.0 in a glass test tube. Equal volumes of euglobulins, fibrinogen at 1 mg/ml, and thrombin (type 1, Sigma) at 50 units/ml were added together.

Platelet aggregation was done two hours after blood collection on a Payton aggregation module, model 600 (Payton Associates, Inc.; Buffalo, NY), with an

Omniscribe<sup>R</sup> chart recorder (Houston Instrument; Austin, TX). Cuvettes of 7.92 millimeter diameter were treated with Prosil<sup>R</sup>-28, an organosilane reagent, according to the manufacturer's instructions (SCM Corporation; Gainesville, FL). Stirring speed was 900 RPM, and temperature was 37°C. The platelet count was adjusted to 200,000/mm<sup>3</sup>. Adenosine diphosphate (ADP) and collagen were obtained from Bio/Data Corporation (Horsham, PA). To 0.225 ml of platelet poor plasma was added 0.025 ml of ADP or collagen. The final concentration in the cuvette for ADP was  $2.0 \times 10^{-5}$  M, and for collagen it was 0.26 mg/ml. Quantitation of platelet aggregation was done according to Newhouse and Clark (29).

Preparation of platelets for scanning electron microscopy followed the procedure of Mattson *et al.* (26). The coverslips for examination were critical point dried, mounted on stubs, and sputter coated with gold-palladium. They were viewed on an AMR model 1000A scanning electron microscope (Lico, Inc.; Bedford, MA).

Statistical Analysis. Statistical analysis for the data was done by the Dunnett's test, which compares a control group versus experimental groups (7). The control group consisted of the results of the two control weeks and the results from the probe insertion done at room temperature. No statistically significant difference in results was noted for these three weeks. The experimental groups comprised the 1 hour, 24 hour, 48 hour, and the 72 hour results. For the platelet aggregation studies the control group consisted of the one week control results and the experimental groups which were similar to those mentioned above.

## RESULTS

Injury Production. Figure 1 shows a freezing and rewarming cycle of a representative rabbit foot from each of the two temperature groups. For the 25 rabbits cooled to  $-20^{\circ}\text{C}$ , the average time for freezing was 22.0 minutes (range 10.0-35.5 minutes) and for rewarming, 12.0 minutes (range 7.0-13.5 minutes). The average temperature from the rectum was  $38^{\circ}\text{C}$  (range  $36.5^{\circ}\text{C}$ - $42.0^{\circ}\text{C}$ ); from the skin,  $35.4^{\circ}\text{C}$  (range  $31.0^{\circ}\text{C}$ - $41.9^{\circ}\text{C}$ ); and from the room,  $23.5^{\circ}\text{C}$  (range  $21.0^{\circ}\text{C}$ - $26.6^{\circ}\text{C}$ ). For the 12 rabbits cooled to  $+5^{\circ}\text{C}$ , the average time for freezing was 13.0 minutes (range 5.0-17.0 minutes) and for rewarming, 5.0 minutes (range 3.5-8.0 minutes). The average temperature from the rectum was  $38.1^{\circ}\text{C}$  (range  $37.4^{\circ}\text{C}$ - $38.8^{\circ}\text{C}$ ); from the skin,  $36.5^{\circ}\text{C}$  (range  $35.2^{\circ}\text{C}$ - $37.5^{\circ}\text{C}$ ); and from the room,  $23.7^{\circ}\text{C}$  (range  $20.3$ - $26.7^{\circ}\text{C}$ ). The temperatures of each rabbit from the groups varied about one degree during the experiment.

Injury Description. For the rabbits cooled to  $-20^{\circ}\text{C}$ , there was at 1 hour after rewarming excessive swelling and reddening of the frozen area with some seepage of fluid at different points from the injured area. At 24 hours the lower one-third area of the metatarsal and the entire area of the phlanges were excessively swollen and dark reddish blue in color. The ventral skin surface of the phlanges showed blisters or cracking of the skin. By 48 hours to 72 hours the frozen area was moderately to excessively swollen and dark red in color. In some rabbits the nails were lost, and there was ulceration of the tips of those phlanges. By 2 weeks the entire area of the phlanges and the distal one-third to one-half of the metatarsal area was severely ulcerated, mummified, or self-amputated.

In rabbits cooled to  $+5^{\circ}\text{C}$ , there was moderate swelling of the cooled area, and the skin color was unchanged at 1 hour after rewarming. At 24 hours the phlanges were moderately swollen and reddish blue in color. The metatarsal area

was slightly swollen with normal color. By 48 to 72 hours there was moderate swelling of the phlanges and loss of some of the nails. Necrosis of the tissue was beginning at the very tip of one or several phlanges. At 2 weeks there was no swelling, and the metatarsal region and most of the phlanges were normal in color. At the tip of the phlanges that were damaged, the scab was usually entirely lost, and the underlying tissue appeared shinny and pinkish red in color with very little swelling.

Blood Changes. The changes observed in the blood cells and certain blood proteins in severe frostbite injury at the different time intervals are shown in Table 1. At 1 hour after injury plasma hemoglobin significantly increased. At 24 hours the leukocyte count and the plasma fibrinogen concentration increased, while the plasma albumin concentration decreased. At this time the plasma hemoglobin concentration returned to the control value. At 48 hours the leukocyte count and the fibrinogen and albumin concentrations were changed as for the 24 hours. During this time the platelet count and the antithrombin-III activity were elevated significantly. The change in the leukocytes was due to a decrease in the number of lymphocytes and an increase in the number of neutrophiles. At 72 hours the leukocyte, neutrophile, and platelet counts; fibrinogen concentration; and the antithrombin-III activity were elevated. The erythrocyte and lymphocyte counts, hematocrit, and the albumin concentration were decreased. No change was observed in the serum globulin concentration during any of the time periods.

In Table 2 are the results for the changes in blood cells and certain blood proteins in mild frostbite injury. At 1 hour after injury the plasma hemoglobin concentration increased. This returned to the control level at 24 hours. At this time there were no significant changes in any of the measured items. At 48 hours the lymphocyte count decreased, and the neutrophile count increased

significantly, but there was no significant change in the total leukocyte count. The erythrocyte count and the hematocrit decreased, and the fibrinogen concentration increased significantly. At 72 hours the leukocyte and neutrophile counts and the fibrinogen concentration increased, whereas the erythrocyte and lymphocyte counts and the hematocrit decreased. The difference between the severe and the mild frostbite injury at 72 hours after injury was that only severe frostbite injury showed significant changes in the platelet count, plasma albumin concentration, and the antithrombin-III activity, whereas mild frostbite showed no change in these components. As in the severe frostbite injury no change was observed in the serum globulin concentration in mild frostbite injury.

Plasma coagulation results after severe frostbite injury are shown in Table 3. No significant changes in any of the clotting assays occurred 1 hour after rewarming. After 24 hours only the prothrombin time was shortened significantly. By 48 hours the prothrombin time still was shortened, and the activities of the clotting Factors XI, X, VII were elevated. 72 hours after injury the activated partial thromboplastin time was prolonged. The prothrombin time was shortened, and the activities of the clotting Factors XI, X, IX, and VII were elevated significantly. Clotting Factors XII, VIII, and II showed no change in their activities during any of the times of observation. No clotting factor showed a significant decreased in its activity at any of the time periods.

Table 4 shows the plasma coagulation results for mild frostbite injury. No significant changes occurred in any of the clotting assays at 1 hour after rewarming. By 24 hours only Factor XII showed a momentary significant decrease in its activity, which returned to the control level for the remaining observation periods. Factor IX activity was elevated at 48 hours, but its activity returned to the control level by 72 hours. After 72 hours the activated partial thromboplastin time was prolonged, and Factor VII activity was elevated

significantly. Compared to severe frostbite injury after 72 hours, there were fewer changes in clotting assays observed in mild frostbite injury.

Changes in the fibrinolytic activity were measured for five severe and five mild frostbitten rabbits. No significant changes were observed between the control and the experimental results in either rabbit group in the euglobulin lysis time test (range 218-350 minutes), plasminogen assay (range 63-94 nmoles tyrosine  $\text{min}^{-1} \text{ml}^{-1}$ ), and the fibrinogen/fibrin degradation product assay by the staphylococcal clumping method (range 0-3  $\mu\text{g/ml}$ ), by the protamine sulfate method (zero range), and by the tanned red cell hemagglutination inhibition method (zero range).

Platelet aggregation induced by adenosine diphosphate and collagen was studied only in severely frostbitten rabbits. Table 5 shows that there was only a significant increase in the slope of the collagen induced platelet aggregation results at 24 hours, 48 hours, and 72 hours after rewarming. Scanning electron micrographs showed no changes in the appearance of the platelets after frostbite injury.



## DISCUSSION

In this study we tried to determine the changes that occur in the blood of rabbits during the acute stage of frostbite. Much of our information regarding the pathophysiology of frostbite has come from experimental lesions in the rabbit. The experimental frostbite in the rabbit is qualitatively and possibly quantitatively similar to human ground type frostbite (30).

Chohan et al. (8) concluded from 15 cases of frostbite among soldiers at high altitude that disseminated intravascular coagulation occurred in frostbite injury. Barkagan and Plotnikov (2) studied acute frostbite injury of the extremities in 154 people with first and second degree frostbite injury and in 106 people with third and fourth degree frostbite injury. All of their patients showed an increase in the degree of platelet aggregation caused by adenosine diphosphate and thrombin, and activation of the contact phase of coagulation, and an acceleration of the extrinsic clotting pathway. They found that a complex disaggregating-anticoagulant therapy produced very favorable results in treating patients with acute frostbite.

In our study we found that there was no evidence of disseminated intravascular coagulation in rabbits having frostbite injury. These rabbits had no hypofibrinogenemia, no increased serum fibrinogen/fibrin degradation products, no decreased plasma levels of clotting factors, no thrombocytopenia, and no decreased antithrombin-III activity. In disseminated intravascular coagulation these changes would occur.

The results that we obtained in the rabbit have been observed in certain clinical conditions. We observed a decrease in the hematocrit of the rabbit after frostbite injury. Gruner (17) reported a similar finding in rabbits after trauma due to edema, hematoma, contusion, hemorrhage, and leg fracture. In frostbite injury there is inflammation at the site of injury (18). Due to this we observed

an increase in leukocytes in both severe and mild frostbite injury. The increase in the number of platelets that we observed in the severe frostbite injury has been observed in various human diseases or conditions, such as, iron deficiency, inflammation, malignant diseases, splenectomy, rheumatoid arthritis, and thromboembolic disorders (11,20,35).

The increase in the fibrinogen concentration and the antithrombin-III activity and the decrease in the albumin concentration in the frostbite injury in the rabbit are due to the fact that these proteins are acute phase reactant proteins (33). Fibrinogen and antithrombin-III are positive acute phase reactant proteins whose plasma concentration increases due to increased liver synthesis in response to inflammation, whereas albumin is a negative acute phase reactant protein whose plasma concentration decreases in response to inflammation. Murazyan et al. (28) found an elevated fibrinogen concentration in their human frostbite cases, but Chohan et al. (8) found in their human cases decreased concentrations of fibrinogen and albumin and a decreased antithrombin-III activity 24 hours after frostbite injury.

Both severe and mild frostbitten rabbits had hemolysis 1 hour after rewarming. This hemolysis was due to the freezing and the subsequent thawing of the frozen tissue.

Although in dogs (31) and in monkeys (5), infusion of autologous hemolysate caused a hypercoagulable state, this did not occur in rabbits injected with moderately large quantities of hemolysate (38). This difference could be due to a specific species variance or to the quantity of the hemolysate.

In the present study we saw in severe frostbite an increase in the level of Factor I and in the activities of Factors VII, IX, X, and XI, whereas in mild frostbite injury we saw a rise in the activities of Factors VII and IX. An increase in the activities of various clotting factors has been seen in a number of clinical

situations. In patients surviving from myocardial infarction there was seen an increase in the levels of Factors I, VIII, IX, X, and XI (16,32). After trauma due to a fracture of the long bone or after giving birth, Davidson and Tomlin (9) observed in these two groups an increase in the activities of Factors I, VIII, IX, and X. In addition the activities of Factors V and VII were increased only in the puerperal group. Following major surgery Factors VIII, IX, and XI have been reported to increase (14). From these observations it can be concluded that the body responds to various physical stresses by increasing certain clotting factors.

The acceleration of the prothrombin time in frostbite in rabbits indicated that there was an activation of the extrinsic clotting pathway. We do not have an explanation for the prolongation of the partial thromboplastin time when there was no deficiency of a measured clotting factor in the extrinsic, intrinsic, or common pathways. Experiments mixing control plasma with plasma after frostbite injury indicated that a deficiency and not an inhibitor was present in the intrinsic pathway of the frostbitten rabbit.

No change in the fibrinogen/fibrin degradation products (FDP) occurred after frostbite injury in the rabbit. Spector et al. (38) found no FDP in their control rabbits in experiments on hemoglobinemia. An increase in FDP would indicate that intravascular coagulation is occurring. However, the rabbit shows a slight increase or no increase in FDP during induced intravascular coagulation (37). Hedlin et al. (22) concluded that the rat, rabbit, and dog have a fibrinolytic mechanism that does not bear a similarity to human fibrinolysis because the animal blood compared to human blood has a high level of antifibrinolytic activity. Also the rabbit has a significantly low concentration of plasminogen compared to human blood (37).

Changes in platelet aggregation after frostbite injury differ in rabbit and in humans. The only change measured in the rabbit was an increase in the slope

of collagen induced platelet aggregation. No change was seen when using adenosine diphosphate as the aggregator. Barkagan and Plotnikov (2) observed for adenosine diphosphate and thrombin induced platelet aggregation in human frostbite injury an increase in the reaction time and in the percent change in light transmission. Both of these changes were related to the severity of the human frostbite injury.

The results of our study indicate that the blood changes reported for human frostbite injury are different from what is observed in the rabbit model. Both human and rabbit frostbite show an increase in fibrinogen and an activation of the extrinsic pathway of coagulation, but only disseminated intravascular coagulation occurs in human frostbite injury. There is a species difference in response to frostbite injury.

## SUMMARY

Acute systemic blood changes were measured in New Zealand white rabbits after severe and mild frostbite injury to the foot. There was observed after 72 hours in the severely frostbitten rabbits a decrease in erythrocytes, hematocrit, lymphocytes, and albumin and an increase in total leukocytes, neutrophils, platelets, fibrinogen, and antithrombin-III. Mild frostbitten rabbits showed similar changes except for no changes in the platelets, albumin, and antithrombin-III. In severely frostbitten rabbits after 72 hours the changes in the plasma coagulation tests were a prolonged partial thromboplastin time, an accelerated prothrombin time and increased activities of Factors VII, IX, X, and XI. In mild frostbitten rabbits there were a prolonged partial thromboplastin time and an increased activity of Factor VII. No changes in fibrinolysis were seen in either group of rabbits. Platelet aggregation, studied only in the severely frostbitten rabbits, showed a change only by an increase in the slope of the collagen induced platelet aggregation. The blood changes observed in the rabbit model are different than those reported in human frostbite cases. No disseminated intravascular coagulation was apparent in the rabbit model after frostbite injury.

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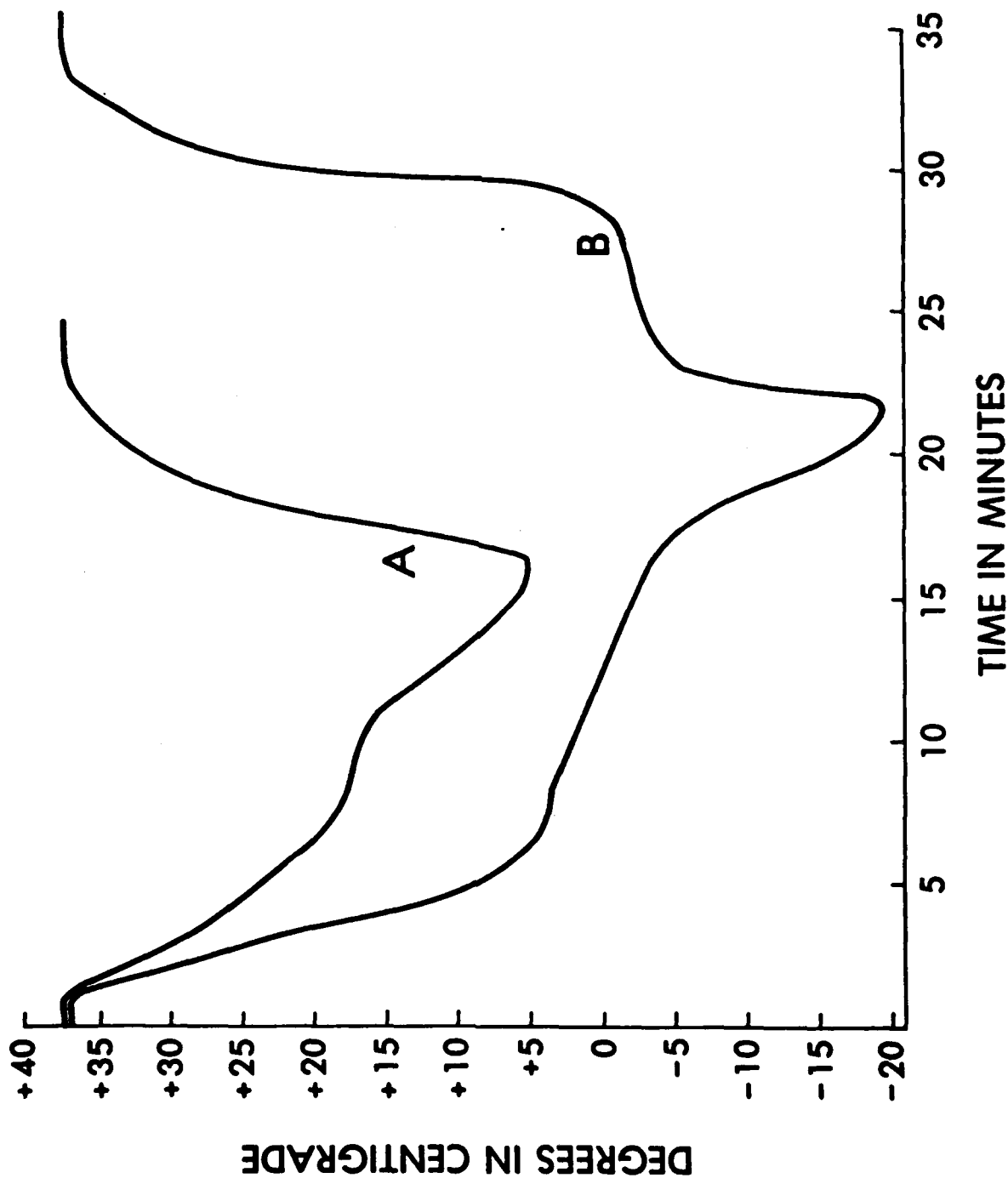


FIGURE 1: REPRESENTATIVE CURVES FOR THE FREEZING AND REWARMING CYCLE OF A RABBIT FOOT WHICH REACHED A LOW TEMPERATURE OF  
A) +5°C OR B) -20°C

Table 1

Changes in Blood Cells and Blood Proteins  
in Severe Frostbite Injury

Item	Number of Rabbits	Control	Time After Injury			
			1 hour	24 hours	48 hours	72 hours
Erythrocyte ( $10^6/\text{mm}^3$ )	25	5.22 $\pm$ 0.10*	5.68 $\pm$ 0.10	5.25 $\pm$ 0.17	4.66 $\pm$ 0.16	4.33 $\pm$ 0.15**
Leukocyte ( $10^3/\text{mm}^3$ )	25	8.27 $\pm$ 0.41	9.35 $\pm$ 0.84	13.98 $\pm$ 0.86**	16.85 $\pm$ 1.50**	19.82 $\pm$ 1.99**
Lymphocyte (%)	9	71 $\pm$ 2	57 $\pm$ 5	54 $\pm$ 4**	51 $\pm$ 4**	48 $\pm$ 4**
Neutrophile (%)	9	24 $\pm$ 8	38 $\pm$ 13	37 $\pm$ 12	45 $\pm$ 15	48 $\pm$ 16
Platelet ( $10^3/\text{mm}^3$ )	21	4.03 $\pm$ 0.16	4.19 $\pm$ 0.25	4.28 $\pm$ 0.29	5.14 $\pm$ 0.31**	5.18 $\pm$ 0.29**
Hematocrit (%)	25	33.4 $\pm$ 0.6	36.6 $\pm$ 0.7	33.2 $\pm$ 1.0	29.7 $\pm$ 1.0**	28.1 $\pm$ 1.0**
Plasma Hemoglobin (mg/100ml)	23	1.57 $\pm$ 0.11	3.40 $\pm$ 0.26**	1.99 $\pm$ 0.19	1.48 $\pm$ 0.13	1.39 $\pm$ 0.11
Fibrinogen (mg/100ml)	13	176.3 $\pm$ 11.5	203.4 $\pm$ 13.1	294.2 $\pm$ 22.5**	333.6 $\pm$ 40.3**	399.2 $\pm$ 46.6**
Albumin (gm/100ml)	9	4.10 $\pm$ 0.11	3.78 $\pm$ 0.23	3.48 $\pm$ 0.13**	3.43 $\pm$ 0.11**	3.54 $\pm$ 0.12**
Globulin (gm/100ml)	5	2.14 $\pm$ 0.08	2.27 $\pm$ 0.10	2.13 $\pm$ 0.17	2.06 $\pm$ 0.17	2.10 $\pm$ 0.17
Antithrombin-III (%)	9	100.0	96.6 $\pm$ 5.2	100 $\pm$ 3.7	115.9 $\pm$ 5.5**	130.6 $\pm$ 9.4**

\* Mean  $\pm$  Standard Error

\*\* Probability &lt; 0.05

Table 2

Changes in Blood Cells and Blood Proteins  
in Mild Frostbite Injury

Item	Number of Rabbits	Control	Time After Injury			
			1 hour	24 hours	48 hours	72 hours
Erythrocyte ( $10^6/\text{mm}^3$ )	6	4.91 $\pm$ 0.19*	4.92 $\pm$ 0.21	4.57 $\pm$ 0.36	4.33 $\pm$ 0.34**	4.10 $\pm$ 0.25**
Leukocyte ( $10^3/\text{mm}^3$ )	6	10.40 $\pm$ 0.49	7.92 $\pm$ 0.60	12.83 $\pm$ 1.46	13.72 $\pm$ 0.99	16.85 $\pm$ 2.63**
Lymphocyte (%)	6	69 $\pm$ 3	51 $\pm$ 7	59 $\pm$ 6	44 $\pm$ 10**	48 $\pm$ 8**
Neutrophile (%)	6	28 $\pm$ 11	40 $\pm$ 16	37 $\pm$ 15	52 $\pm$ 21**	49 $\pm$ 20**
Platelet ( $10^3/\text{mm}^3$ )	6	3.88 $\pm$ 0.33	3.52 $\pm$ 0.40	4.17 $\pm$ 0.29	3.90 $\pm$ 0.42	4.07 $\pm$ 0.34
Hematocrit (%)	6	32 $\pm$ 0.8	32.4 $\pm$ 1.2	30.1 $\pm$ 2.2	29.3 $\pm$ 2.1**	28.0 $\pm$ 1.3**
Plasma Hemoglobin (mg/100ml)	6	1.45 $\pm$ 0.14	2.34 $\pm$ 0.45**	1.57 $\pm$ 0.18	1.73 $\pm$ 0.27	1.18 $\pm$ 0.21
Fibrinogen (mg/100ml)	6	166.5 $\pm$ 20.7	175.5 $\pm$ 38.9	235.0 $\pm$ 47.6	281.2 $\pm$ 51.6**	237.0 $\pm$ 66.1**
Albumin (gm/100ml)	6	3.84 $\pm$ 0.24	3.75 $\pm$ 0.16	3.63 $\pm$ 0.19	3.70 $\pm$ 0.28	3.77 $\pm$ 0.21
Globulin (gm/100ml)	5	2.41 $\pm$ 0.07	2.33 $\pm$ 0.11	2.47 $\pm$ 0.09	2.45 $\pm$ 0.12	2.44 $\pm$ 0.10
Antithrombin-III (%)	6	100.0	101.5 $\pm$ 4.0	96.0 $\pm$ 6.2	113.0 $\pm$ 5.4	108.7 $\pm$ 3.1

\* Mean  $\pm$  Standard Error

\*\* Probability &lt; 0.05

Table 3

Changes in the Coagulation Tests and  
Factor Assays in Severe Frostbite Injury

## Time After Injury

Item	Number of Rabbits	Control	Time After Injury			
			1 hour	24 hours	48 hours	72 hours
Activated Partial Thromboplastin Time (sec)	9	38.9 ± 3.3*	42.9 ± 4.6	44.0 ± 3.4	42.8 ± 5.2	52.0 ± 4.0**
Prothrombin Time (sec)	9	7.0 ± 0.2	7.0 ± 0.2	6.7 ± 0.2**	6.4 ± 0.2**	6.3 ± 0.2**
Factor XII (%)	9	100.0	100.7 ± 7.1	100.2 ± 7.6	112.4 ± 8.9	113.4 ± 5.7
Factor XI (%)	9	100.0	91.3 ± 8.9	89.2 ± 6.4	144.6 ± 26.2**	139.2 ± 16.7**
Factor X (%)	9	100.0	93.1 ± 5.6	132.0 ± 8.2	166.7 ± 18.2**	168.1 ± 13.7**
Factor IX (%)	9	100.0	76.4 ± 4.8	97.4 ± 9.4	144.3 ± 10.3	164.9 ± 7.7**
Factor VIII (%)	9	100.0	82.9 ± 5.8	103.1 ± 7.8	123.4 ± 9.5	119.0 ± 11.5
Factor VII (%)	9	100.0	88.2 ± 8.0	124.7 ± 10.2	164.9 ± 24.4**	161.1 ± 19.3**
Factor II (%)	5	100.0	106 ± 9.8	99.6 ± 8.0	97.0 ± 7.0	103.6 ± 8.4

\*Mean ± Standard Error

\*\* Probability &lt; 0.05

Table 4

## Changes in the Coagulation Tests and Factor Assays in Mild Frostbite Injury

Item	Number of Rabbits	Control	Time After Injury			
			1 hour	24 hours	48 hours	72 hours
Activated Partial Thromboplastin Time (sec)	5	34.5 ± 5.4*	28.8 ± 4.2	35.7 ± 9.5	35.6 ± 7.1	46.7 ± 11.4**
Prothrombin Time (sec)	6	6.7 ± 0.1	6.8 ± 0.1	6.5 ± 0.1	6.4 ± 0.2	6.5 ± 0.1
Factor XII (%)	6	100.0	97.5 ± 8.2	81.7 ± 6.5**	96.7 ± 8.1	102.3 ± 6.7
Factor XI (%)	6	100.0	90.3 ± 9.9	87.3 ± 5.4	99.7 ± 6.3	89.7 ± 4.6
Factor X (%)	6	100.0	106.2 ± 9.2	130.7 ± 11.3	147.3 ± 17.7	144.3 ± 21.1
Factor IX (%)	6	100.0	101.5 ± 9.8	104.0 ± 7.6	123.3 ± 13.0**	118.8 ± 20.4
Factor VIII (%)	6	100.0	98.2 ± 9.2	86.5 ± 4.1	98.0 ± 7.4	111.8 ± 10.3
Factor VII (%)	6	100.0	112 ± 14.4	122.3 ± 10.5	144.0 ± 22.4	162.0 ± 24.5**
Factor II (%)	6	100.0	95.2 ± 8.8	95.8 ± 6.5	99.2 ± 10.8	102.0 ± 15.2

\*Mean ± Standard Error

\*\* Probability &lt; 0.05

Table 5

Changes in Platelet Aggregation  
in Severe Frostbite Injury

Item	Number of Rabbits	Control	Time After Injury			
			1 hour	24 hours	48 hours	72 hours
ADP Reaction Time (sec)	7	4.4 ± 0.1*	4.9 ± 0.2	4.7 ± 0.1	4.5 ± 0.3	4.2 ± 0.3
ADP Aggregation	7	6.4 ± 0.5	5.5 ± 0.3	5.3 ± 0.6	5.0 ± 0.3	5.6 ± 0.2
ADP Disaggregation Slope	7	2.2 ± 0.3	2.3 ± 0.3	1.9 ± 0.3	1.5 ± 0.2	1.7 ± 0.2
ADP Maximum Aggregation (%)	7	32.5 ± 3.1	28.2 ± 3.1	28.3 ± 4.0	29.0 ± 4.0	32.7 ± 3.1
Collagen Reaction Time (sec)	6	215 ± 29	249 ± 46	250 ± 51	258 ± 40	269 ± 38
Collagen Aggregation Slope	6	1.1 ± 0.3	0.8 ± 0.2	0.6 ± 0.1**	0.6 ± 0.2**	0.7 ± 0.2
Collagen Maximum Aggregation (%)	6	51.7 ± 5.8	43.3 ± 5.9	38.8 ± 4.2	40.8 ± 6.3	41.8 ± 8.5

\*Mean ± Standard Error

\*\* Probability &lt; 0.05



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